

# Karyotype description and comparative analysis in Ringed Kingfisher and Green Kingfisher (Coraciiformes, Alcedinidae)

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## Abstract

Kingfishers comprise about 115 species of the family Alcedinidae, and are an interesting group for cytogenetic studies, for they are among birds with most heterogeneous karyotypes. However, cytogenetics knowledge in Kingfishers is extremely limited. Thus, the aim of this study was to describe the karyotype structure of the Ringed Kingfisher (*Megaceryle torquata* Linnaeus, 1766) and Green Kingfisher (*Chloroceryle americana* Gmelin, 1788) and also compare them with related species in order to identify chromosomal rearrangements. The Ringed Kingfisher presented  $2n = 84$  and the Green Kingfisher had  $2n = 94$ . The increase of the chromosome number in the Green Kingfisher possibly originated by centric fissions in macrochromosomes. In addition, karyotype comparisons in Alcedinidae show a heterogeneity in the size and morphology of macrochromosomes, and chromosome numbers ranging from  $2n = 76$  to 132. Thus, it is possible chromosomal fissions in macrochromosomes resulted in the increase of the diploid number, whereas chromosome fusions have originated the karyotypes with low diploid number.

## Keywords

Aves, chromosome, evolution, karyotype

## Introduction

Avian karyotypes are characterized by internal variation in the size of chromosomes, presenting two distinct groups, macrochromosomes and microchromosomes. About eight pairs of macrochromosomes are seen in most of birds, and the remaining are microchromosomes (Rodionov 1996). Diploid number also varies, including species with a low diploid number such as *Burhinus oedicephalus* Linnaeus, 1758 (Charadriiformes)  $2n = 40$  (Nie et al. 2009), and high  $2n = 136-142$  in *Corythaixoides concolor* Smith, 1833 (Musophagiformes) (Christidis 1990), but most of the species exhibit karyotypes with  $2n = 74-86$  (Tegelstrom and Rytman 1981).

Studies of karyotype structure in birds have given valuable information about evolutionary relationships. Chromosome painting shows that, although relatively conserved, the macrochromosomes evolve through several intra and inter-chromosomal rearrangements (de Oliveira et al. 2010, Kretschmer et al. 2014). While Tandem fusions between microchromosomes and micro- with macrochromosomes have resulted in decrease of diploid number (Nishida et al. 2008, Nie et al. 2009, de Oliveira et al. 2010, 2013). Chromosome fission in recurrent breakpoints has been documented in macrochromosomes, and can result in increase of chromosome number (Skinner and Griffin 2012, Degrandi et al. 2017).

In relation to the sex chromosomes of birds, males have a homogametic ZZ pair and female have a heterogametic ZW (Schartl et al. 2015). The Z chromosome is a highly conserved macrochromosome and it comprises 7% of the haploid genome (Graves and Shetty 2001). In Piciformes, Bucerotiformes, and Coraciiformes the Z chromosome is often the largest chromosome of the complement (de Oliveira et al. 2017). Whereas the W chromosome is highly variable in size, and has been observed from homomorphic to Z in Paleognaths Ratite (Nishida-Umehara et al. 2007) to a small and heterochromatic with variable size in Neognaths birds (Graves and Shetty 2001). This size variation has been attributed to a differential accumulation and degradation of repetitive DNAs (de Oliveira et al. 2017). Also, a multiple sex chromosome system was recently described for the Adelie Penguin (*Pygoscelis adeliae* Hombron et Jacquinet, 1841/ Sphenisciformes) where males have  $Z_1Z_1Z_2Z_2$  and females  $Z_1Z_2W$  (Ganski et al. 2017).

Kingfishers (Alcedinidae) comprises a diverse family of birds with approximately 115 species distributed worldwide (Gill and Donsker 2017). They are an interesting group for cytogenetic studies since they are among birds with most heterogeneous karyotypes. However, knowledge about cytogenetics in Kingfishers is extremely limited. There are records for *Dacelo novaeguineae* Hermann, 1783,  $2n = 76$ , *Halcyon smyrnensis* Linnaeus, 1758,  $2n = 76$ , *Halcyon pileata* Boddaert, 1783,  $2n = 84$ , *Alcedo atthis* Linnaeus, 1758,  $2n = 132$ , *Ceyx azureus* Latham, 1801,  $2n = 122$ , and *Ceryle rudis* Linnaeus, 1758,  $2n = 82$  (De Boer and Belterman 1980, Xiaozhuang and Qingwei 1989, Christidis 1990, Youling et al. 1998, Garg and Shrivastava 2013).

The Ringed Kingfisher, *Megaceryle torquata* Linnaeus, 1766 and the Green Kingfisher, *Chloroceryle americana* Gmelin, 1788 belong to subfamily Cerylinae and their

karyotypes are unknown (Moyle 2006). In view of this, the present study aimed to describe the karyotype structure of these species. Secondly, we sought to gather karyotype information from Alcedinidae in order to compare them and to identify the chromosomal rearrangements.

## Material and methods

### Samples and location

The karyotype of one male and one female of *Megaceryle torquata* (Fig. 1A) collected at the Parque Ecológico El Puma in Argentina, and two males and one female of *Chloroceryle americana* (Fig. 1C) from Santa Maria/Rio Grande do Sul, Brazil were analyzed for this work. Specimens were collected according to license SISBIO 44173-1 and animal research ethics committee (CEUA 018/2014).

### Cell culture

Mitotic chromosomes in *M. torquata* specimens were obtained by lymphocyte culture according to Moorhead et al. (1960). In short, blood samples were incubated in medium PBMax (Gibco) for 72 hours at 38 °C. In the last hour of incubation, 0,001 ml of colchicine solution (0.05%) was added. After these procedures, the cells were centrifuged and pellet was incubated in 10 mL of hypotonic solution (0.075 M KCl) for 20 min, followed by fixation in three washes with Methanol: Acetic acid 3:1 solution.

In *C. americana*, mitotic cells were obtained from bone marrow according to Garnero and Gunski (2000). Initially, bone marrow was extracted from femurs and incubated in a 10 ml of RPMI 1640 medium with 0,001 ml of colchicine solution (0.05%) at 39 °C for 1 hour. Finally, cells were incubated in 10 ml of hypotonic solution (0.075 M KCl) for 20 minutes. Then cells were washed three times with Methanol: Acetic acid 3:1 solution.

### Chromosomal analyses

The diploid number was determined by analyzing approximately 40 metaphases per specimen, by conventional 0,8% Giemsa staining solution. Karyotypes were organized according to chromosome size and differential staining CBG-banding (Sumner 1972) was applied to identify the W chromosome.

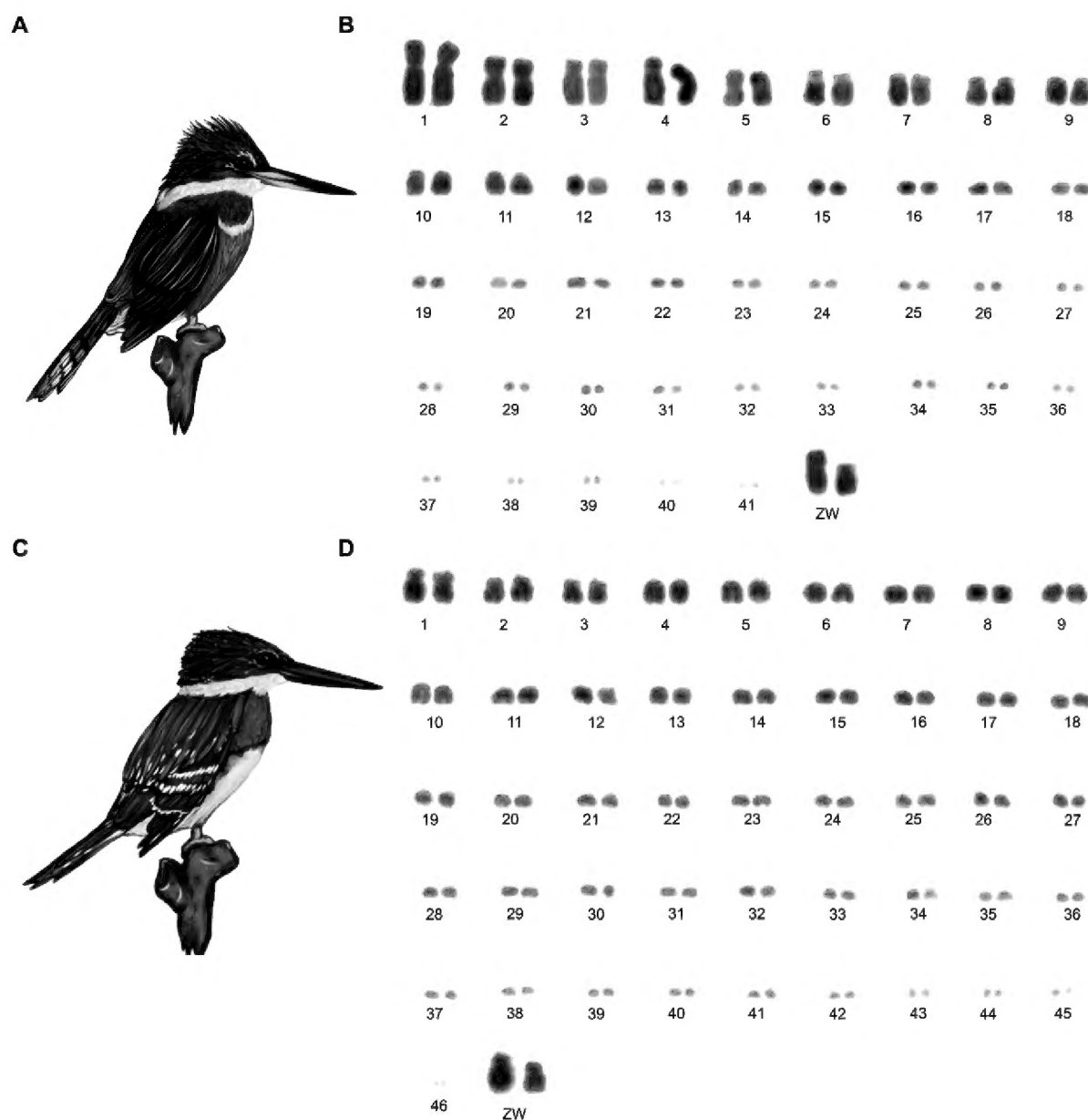
Morphometry of the first 15 autosomal chromosomes pairs and the ZW sex chromosomes, were performed in Alcedinidae species available. Centromeric index (CI) was estimated by ratio of short arm length by total chromosome length. Nomenclature for chromosome morphology were performed according to Guerra (1986) using CI index.

## Results

The Ringed Kingfisher presented chromosome number of  $2n = 84$  (Figure 1B). The chromosome set is composed of ten biamed pairs, being the submetacentric pairs (1, 3 and 4), metacentric (2, 5, 8 and 13) and acrocentric (6, 7 and 9). The remaining autosomes are telocentric. Z and W are both submetacentric macrochromosomes, with size similar to chromosome 4 and 9, respectively.

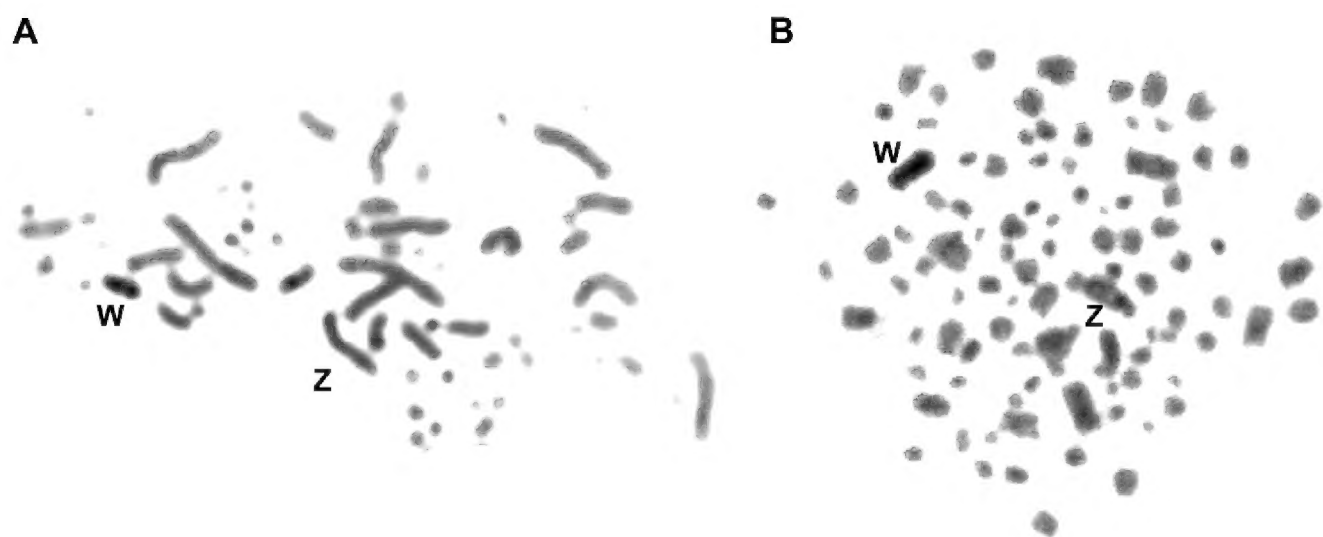
The Green Kingfisher had a diploid number of  $2n = 94$  (Fig.1D), consisting of only four biamed pairs, where 1, 2 and 3 are submetacentric and 12 is metacentric. All the other chromosome pairs are telocentric. The Z chromosome is submetacentric and is the largest chromosome of the karyotype, while the W chromosome is submetacentric with size between 1 and 2.

C-banding analysis allowed correct identification of the W chromosome, since both species presented a highly heterochromatic pattern for this chromosome (Fig. 2A and B). The Z chromosome was euchromatic in both species. However, in *C. americana* a positive staining was observed near the centromere (Fig. 2 B).



**Figure 1.** Ringed Kingfisher *Megasceryle torquata* (A), and karyotype with  $2n = 84$  (B). Green Kingfisher *Chloroceryle americana* (C), and karyotype with  $2n = 94$  (D).

In the literature, chromosome data were found for *C. rudis*, *H. pileata*, *A. atthis*, *H. smyrnensis*, *D. novaeguineae*, and *C. azureus* (Table 1). Unfortunately, for *H. smyrnensis*, *D. novaeguineae*, *C. azureus* only the diploid number was available. Despite this, some observations can be made: i) diploid number is highly variable; ii) number of biarmed chromosomes (metacentric, submetacentric, and acrocentric) was also variable; iii) the Z chromosome is a conserved submetacentric chromosome; and iv) the W chromosome morphology is variable among species, appearing as metacentric or submetacentric.



**Figure 2.** Comparative C-banding analysis of the Ringed Kingfisher *Megaceryle torquata* (A) and the Green Kingfisher *Chloroceryle americana* (B).

**Table 1.** Karyotype information's in Alcedinidae species.

Species	2n	Nº biarmed	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Z	W	Reference
<i>Chloroceryle americana</i>	94	4	S	S	S	T	T	T	T	T	T	T	T	M	T	T	T	S	S	Present work
<i>Ceryle rudis</i>	82	13	M	M	M	M	M	M	S	S	A	A	A	A	A	T	T	S	M	Garg and Shrivastava 2013.
<i>Megaceryle torquata</i>	84	10	S	M	S	S	M	A	A	S	A	T	T	T	M	T	T	S	S	Present work
<i>Halcyon pileata</i>	84	12	M	M	S	S	M	M	M	S	T	T	M	T	M	M	S	S	M	Xiaozhuang and Qingwei 1989.
<i>Halcyon smyrnensis</i>	76	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Youling et al. 1998.
<i>Dacelo novaeguineae</i>	76	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	De Boer and Belterman 1980.
<i>Alcedo atthis</i>	132	15	M	M	M	S	M	M	M	M	S	M	S	M	M	M	M	S	M	Xiaozhuang and Qingwei 1989.
<i>Ceyx azureus</i>	122	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Christidis 1990.

2n= diploid number; Nº biarmed= Number of biarmed autosomes; Chromosome morphology: (M=metacentric, S=submetacentric, A=Acrocentric and T=Telocentric); - = Not was possible to obtain the information in original work; Species names in accordance to IOC WORLD BIRD LIST (7.3) <http://dx.doi.org/10.14344/IOC.ML.7>.



## Discussion

Unfortunately, forty years after the publication of the karyotype of *D. novaguineae* (*D. gigas* by De Boer and Beltrman 1980), information about cytogenetics of Alcedinidae species is still limited. Nevertheless, comparisons done in this work (Tab. 1) show that Kingfishers present karyotype plasticity, evidenced by variation in diploid number, number of biarmed chromosomes, and in size and morphology of macrochromosomes.

According to White (1977), chromosome fusions result in the reduction of diploid number and increase of number of biarmed chromosomes, while chromosome fissions increase the diploid number and decrease the number of biarmed chromosomes. These mechanisms appear to be adequate to explain the differences in the karyotypes of Alcedinidae species.

In this work, the increasing of diploid number observed in *M. torquata* ( $2n = 84$ ) to *C. americana* ( $2n = 94$ ), (Fig. 1B and D) may have originated by chromosome fissions. Some characteristics support this hypothesis, such as, the number of biarmed chromosomes is reduced from 9 pairs in *M. torquata* for to 4 in *C. americana*, and Z chromosome size is similar to chromosome 4 in *M. torquata*, while in *C. americana*, the Z chromosome is the largest in the karyotype. However, experiments with chromosome painting with specific probes could confirm these hypotheses.

According to Graves and Shetty (2001) Z chromosome size is conserved in most birds. So, Z chromosome size in relation to other macrochromosomes can be considered as a marker for size and evidence of occurrence of chromosome fission or fusions. Chromosome W in *M. torquata* and *C. americana* did not present differences and shows a pattern of heterochromatinization, similar of what has been observed in other Neognaths species. However, when compared to other species of Kingfishers, it is observed that there is a variation in chromosome morphology, ranging from metacentric to submetacentric.

## Conclusion

Kingfishers present interesting chromosomal characteristics. These species have a diploid number which is highly variable and probably originated by fusions and/or fissions involving macrochromosomes. Hence rearrangements in macrochromosomes result in size and morphology variations, characterizing an intra-familial karyotypic heterogeneity. Absence of G-banding pattern and chromosome painting data did not allow comparisons. Therefore, we hope that this work may encourage the development of other cytogenetic studies in Kingfishers, and that our hypothesis of fission and chromosomal fusions as mechanisms responsible for karyotypes differentiation in Kingfishers can be confirmed.

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